Journal of Economic Entomology, 2017, 1–10
doi: 10.1093/jee/tox113

M.A. Navarro-Roldán
Department of Crop and Forest Sciences
University of Lleida (UdL)
Rovira Roure Avenue, 191
25198-Lleida (Spain)
Phone: +34 973 702646
E-mail: m.navarro.roldan@gmail.com

ECOTOXICOLOGY

Comparative effect of three neurotoxic insecticides with different modes of action on adult males and females of three tortricid moth pests

Miguel A. Navarro-Roldán[1], Jesús Avilla[2], Dolors Bosch[3], Joan Valls[4] and César Gemeno[1].

[1] Department of Crop and Forest Sciences. University of Lleida (UdL). 25198-Lleida, Spain

[2] Department of Crop and Forest Sciences. Agrotecnio. University of Lleida (UdL). 25198-Lleida, Spain

[3] Department of Sustainable Crop Protection. Food and Agriculture Research Institute (IRTA) 25198-Lleida, Spain

[4] Biostatistics Unit. Biomedical Research Institute (IRBLLEIDA). 25198-Lleida, Spain

ABSTRACT

Insecticides are the dominant pest management method in fruit and vegetable crops worldwide due to their quick effect, low cost and relatively easy application, but they bear negative effects on human health and the environment. Insecticide mode of action (MoA), target species and sex are variables that could affect insecticide mortality. We recorded the mortality caused by three neurotoxic insecticides with different modes of action [Chlorpyrifos (organophosphate, acetylcholinesterase inhibitor), λ-Cyhalothrin (pyrethroid, sodium channel modulator) and Thiacloprid (neonicotinoid, nicotinic acetylcholinesterase receptor agonist)] applied topically to adult males and females of three economically important tortricid species [*Cydia pomonella* (L.), *Grapholita molesta* (Busck), and *Lobesia botrana* (Denis & Schiffermüller)] that strongly depend on insecticide use for their control. Concentration and dose-mortality curves were recorded at 24 and 48 hours post application. Large mortality differences between insecticides (maximum 7800-fold for LD_{50}) were followed by much lower, yet important, differences between species (maximum 115-fold), and sexes (maximum 41.5-fold). Significant interactions between the three factors indicate that they are not independent from each other. Interestingly, with the organophosphate Chlorpyrifos, males of the three species were less susceptible than females, which was unexpected since females are larger than males. Higher female sensitivity to organophosphates has been reported previously but only in *G. molesta*, not in other moth species. Our results highlight the importance of taking into account sex in dose-mortality studies with adult moths.

KEY WORDS: dose-response, neurotoxic insecticides, Tortricidae, adult insects, sex differences.

A fundamental aspect of insecticide pest control is determining the optimal quantity of toxicant needed to obtain maximum pest mortality while at the same time minimizing environmental and human impact (Guillette and Iguchi 2012, Guedes *et al*. 2015). Mortality curves are the most common method to assess the relationship between the quantity of toxicant and the level of mortality (Pasquier and Charmillot 2003, Cutler 2013). Both, toxicant mode of action and insect species affect the slope and intercept of mortality curves, but these are not the only variables that affect mortality-curves. Some variables such as insect stage (egg, immature or adult) or development are sometimes examined (Knight 2000, Sáenz-de-Cabezón Irigaray *et al*. 2005, Rodríguez *et al*. 2011), whereas other biological variables, such as sex (Kanga *et al*. 2001, Shearer and Usmani 2001, de Lame *et al*. 2001), or methodological issues, such as the mode of application or time of exposure (Preisler and Robertson 1989), are rarely considered. Because it is challenging to compare many variables in a single experiment, most comparative studies either test several insect species with one toxicant (Vandekerkhove and de Clercq 2004, Nayak and Daglish 2006, Ioriatti *et al*. 2009a), or several types of toxicants on a single species (Zotti *et al*. 2013, Grigg-McGuffin *et al*. 2015, Wu *et al*. 2015). Fewer studies, however, test the effect of several insecticides on different species (Beers *et al*. 2005, Fernandes *et al*. 2016, Rodriguez-Saona *et al*. 2016). In addition, the effect of sex is often neglected.

In the present study we compare the effect of three neurotoxic insecticides with different modes of action (MoA) on adult males and females of three economically important moth species. We focus on the tortricid moths, *Cydia pomonella* (L.), *Grapholita molesta* (Busck) and *Lobesia botrana* (Denis & Schiffermüller), because they are key pests of relevant Mediterranean fruit crops, mainly apples, peaches and grapes, but they also attack other hosts and have a relatively worldwide distribution (Ioriatti *et al*. 2011, Damos *et al*. 2015, Kirk *et al*. 2013). As toxicants we chose three neurotoxic insecticides with different modes of action: Chlorpyrifos, an organophosphate that acts on acetylcholinesterase (AChE), the enzyme that degrades the

neurotransmitter acetyl-choline; λ-Cyhalothrin, a pyrethroid that modulates sodium channels involved in action potential generation; and Thiacloprid, a neonicotinoid that competitively modulates nicotinic acetylcholine receptors (nAChR) at the post-synaptic dendrite (Casida 2009, IRAC 2016). Neurotoxic insecticides act by contact and ingestion and could affect all insect life stages. Thiacloprid and Chlorpyrifos affect larvae and adults of *C. pomonella* (Reyes and Sauphanor 2008), Chlorantraniliprole affect eggs and larvae of *L. botrana* (Ioriatti *et al.* 2009b), several neonicotinoid and organophosphate insecticides affect all insect stages of *C. pomonella* and *G. molesta* (Magalhaes and Walgenbach, 2011); and our three test insecticides affect eggs and larvae of *C. pomonella* (Rodriguez et al. 2011).The three insecticides of our study are recommended by the Spanish Agriculture Ministry to control at least two of the three tortricid species each (MAGRAMA 2015). Neurotoxic insecticides account for 54% of total insecticide sales worldwide (Sparks and Nauen 2015). These insecticides have already been tested in these three moth species to assess resistance and sublethal effects, having larva as a common target insect stage. *C. pomonella* is resistant to Chlorpyrifos (Reyes *et al.* 2011, Rodríguez *et al.* 2011), λ-Cyhalothrin (Mota-Sánchez *et al.* 2008, Rodríguez *et al.* 2011) and Thiacloprid (Rodríguez *et al.* 2011, Cichón *et al.* 2013), but there are not registered resistance cases for these active ingredients in *G. molesta* or *L. botrana*. Sublethal effects have been tested for Chlorpyrifos in *C. pomonella* (Yang *et al.* 2013) and *L. botrana* (Pavan *et al.* 2014), for λ-Cyhalothrin in *C. pomonella* (Yang *et al.* 2013) and *G. molesta* (Jones *et al.* 2011), and for Thiacloprid in *C. pomonella* (Brunner *et al.* 2005) and *G. molesta* (Siegwart *et al.* 2011).

By comparing the effect of insecticides with different MoA across phylogenetically related species, and in both sexes, we hope to gain basic background information for further studies on the physiological mechanisms responsible for insecticide resistance and the effect of sublethal doses. At the same time, the response-mortality curves obtained will provide a diagnostic

methodology to test possible resistance cases in field populations, using adults of the same species and the same insecticides tested in this study.

Materials and Methods

Insects. Susceptible laboratory strains of *C. pomonella*, *G. molesta* and *L. botrana* established from individuals collected in Lleida (Spain), Piacenza (Italy), and La Rioja (Spain), respectively, have been maintained under laboratory conditions for more than 5 years without introduction of wild individuals. Larvae were reared in artificial diet (Ivaldi-Sender 1974) in a rearing room maintained at 25 ± 1 ºC with a 16:8 hour light:dark photoperiod. Pupae were separated by sex and checked daily for adult emergence, except for *C. pomonella* which was sexed at the adult stage, also in a daily basis. Adult body mass was estimated by drying 40 frozen 1-day-old individuals of each sex and species for 2 days at 30°C, and then weighting them individually in an analytical balance (± 0.1 mg precision).

Insecticides. Chlorpyrifos (TraceCERT®, certified reference material, ≈100% (a.i.)), λ-Cyhalothrin (PESTANAL®, analytical standard, ≈100% (a.i.)), and Thiacloprid (PESTANAL®, analytical standard, ≈100% (a.i.) (all from Sigma-Aldrich, Spain), were the active ingredients used in the mortality bioassays. All the dilutions used in the bioassays were prepared from pure compound in at least two different occasions, using acetone (CHROMASOLV®, for HPLC, ≥99.9%. Sigma-Aldrich, Spain) as solvent. Dilutions were stored in 2- or 4-ml acetone-rinsed glass vials at 7ºC. The same stock of acetone used to prepare the dilutions was also used as the negative control treatment.

Mortality bioassays. Newly emerged adults were separated from the pupal cages every day and received the insecticide treatments during the first half of the photophase at 0 to 24 hours post-emergence. Adults were placed individually or in pairs in 10-ml test tubes and received a brief (10 seconds) flow of industrial grade CO_2 which quickly anesthetized them. Immediately after being anesthetized they were placed upside down under the field of view of a stereo microscope. A 1-μl test solution was applied to the ventral thoracic region of each insect with a high-precision, positive displacement, repeatable-dispensing micropipette (Multipette® M4, Eppendorf, Germany), and they were transferred immediately to a 150 ml polypropylene non-sterile clinical sample bottle (57 mm diameter x 73 mm-high). Individuals receiving the same treatment were placed in groups of 3 to 10 in the same bottle. The lid of the bottle was punctured to make 10 holes (1-mm-diameter each) to allow gas exchange, and a 1.5 ml eppendorf containing 10% sugar solution and a cotton plug was placed on the bottom to supply nutrients during the observation period. Bottles with treatment insects were placed in the rearing room.

Mortality was recorded at 24 h and 48 h post-treatment. Adults were observed with the naked eye and scored as alive if they flew or walked apparently unaffected, as moribund if they could barely walk or were laying on the bottom of the bottle but still moved, or as dead if they laid immobile on the bottom of the bottle. Mortality was estimated by adding the number of moribund and dead insects.

To select the final concentrations used in the dose-response curves we started testing 1:10 dilutions ranging from 10 µg to 10 pg per insect, with approximately 20 insects in each dose. High and low limits for each curve were roughly estimated this way and new doses (no less than 5 for each treatment combination) were tested, also with approximately 20 insects per dose, until a reasonable probit fit was obtained for a given curve. Using the predicted values from the probit model we chose six final test concentrations (plus acetone control) for each treatment

combination and tested them with in between 60 and 116 insects per concentration. Tests were performed on groups (i.e., repetitions) of at least 3 insects of the same treatment group (insecticide, dose, sex and species), with different treatments tested each day depending on insect availability, until the desired sample size was achieved. A total of 6,802 insects were used to build the final curves.

Data analysis. All the statistical analyses were run in R software (R Core Team 2016). Weight differences among sex and species were analyzed with ANOVA followed by pairwise comparisons. For the analysis of mortality we run generalized linear models (GLM) with a binomial family and a probit link. For statistical analyses we used only the mortality at 24h as a function of insecticide dose (mortality data adjusted by the average dry body weight of each species and sex, i.e., lethal dose or LD). For discussion purposes we show in supplementary material the mortality at 48h as a function of insecticide dose, and 24h mortality as a function of insecticide concentration.

Three levels of mortality analysis were carried out. First, a global model was built hierarchically to determine the effect of insecticide type, dose, species and sex. We started with the simplest model containing no main effects, and followed it with a model including all main effects (insecticide type, dose, insect species and sex), and then with successive models including all main effects and second-, third-, and fourth-order interactions. For model-selection we used the likelihood ratio test (LRT) and the Akaike Information Criterion (AIC), preferring the model with the lower AIC value of pairs that were significantly differences by LRT.

Secondly, we estimated intercepts, slopes and LDs, and their errors for a) insecticides independently of species and sex, b) species within insecticide, independently of sex, and c) sex within species and insecticide. For this, 13 GLM models were run: one to compare insecticides,

three to compare species within each insecticide, and nine for each insecticide by species combination to estimate the effect of sex. Intercept and slope were extracted directly from the GLM models. To estimate LDs, we run the dose.p() function in the package "MASS" of R (Venables and Ripley 2002) on each of the 13 GLM models.

Finally, we performed pairwise comparisons [at same levels indicated above (a, b, c)], for slope, intercept, and LD_{10}, LD_{50} and LD_{90}. For slope and intercept estimates we run a generalized linear hypothesis test using the glht() function in the package "multcomp" of R (Hothorn *et al.* 2008). For comparison of the LDs we calculated the Z-score of the GLM estimates and errors and obtained a p-value by comparison with the Z-score of the standard Normal curve. R scripts and raw data are available at http://hdl.handle.net/10459.1/57672

Results

As expected, females were heavier than males in all three species, and *C. pomonella* was the heaviest of the three species, whereas males of *G. molesta* and *L. botrana* did not differ from each other, and neither did the females (Table S1).

Hierarchical model selection indicated that the most complex model, which contains all main variables plus second-, third- and fourth-order interactions, provided the best fit to the data (Table 1). This 36-parameter model was significantly different from the next simpler model according to likelihood ratio test (LRT), and also had a lower AIC value. Analysis of deviance for this model (Table 2) showed that the highest contribution of main effects was for insecticide dose ($P < 0.0001$), and insecticide type ($P = 0.019$), whereas neither species nor sex contributed significantly on their own ($P > 0.05$). Nine of the eleven second- to fourth-order interactions

were significant, indicating that the effect of individual variables was strongly dependent on the other variables.

Dose-response curves were constructed using the slope and intercept parameters estimated with the individual probit regression models of each curve (Figure 1, Table 3). Several qualitative features are already noticeable in this graph. A group of six green-color curves located on the left of the graph, which are separated by a gap from the rest of the curves on the right, consist of insects treated with λ-Cyhalothrin. This illustrates that this insecticide is a more potent toxicant than the other two, as the model and pairwise comparisons (see below) confirmed. On the right half of the graph the curves for insects treated with Chlorpyrifos (blue) and Thiacloprid (red) are intermixed over a relatively wide dose-range, with and apparent stronger effect of Chlorpyrifos over Thiacloprid. A distinct feature is the blue (Chlorpyrifos) curve located at around the 100 ng dose on a background of red (Thiacloprid) curves. This Chlorpyrifos curve corresponds to *G. molesta* males and departs from the other Chlorpyrifos curves, including *G. molesta* females, which cluster around a lower dose range. This illustrates a strong effect of sex on insecticide response.

Intercepts, slopes and LD estimates (Table 3) were compared among insecticides, species and sexes producing a total of 103 statistical tests (Tables 4 and 5). To analyze the effect of insecticide (independently of species and sex) the intercept and slope of each resulting curve (including all data points, except acetone controls, for all insects treated with each insecticide), was compared with other insecticide resulting curves. A similar procedure was followed to analyze the effect of species (within insecticide and independent of sex) and sex (for each combination of insecticide and species).

Curves that have the same slope and intercept are considered to be equal, curves that have same slope but different intercept are considered parallel, and all other types of curves are neither

equal nor parallel. In our experiment, equal curves occurred only in the comparison between sexes, in *C. pomonella* treated with Chlorpyrifos and λ-Cyhalothrin, and in *L. botrana* treated with λ-Cyhalothrin (Table 4). Parallel curves were observed only in the comparison between species within insecticide, independently of sex. The curves of the three species treated with Thiacloprid were parallel, and so were the curves of *G. molesta* and *L. botrana* treated with λ-Cyhalothrin and the curves of *C. pomonella* and *L. botrana* treated with Chlorpyrifos. All other curves were neither parallel nor equal. λ-Cyhalothrin had lower intercept and higher slope than the other two insecticides, which did not differ from each other in these parameters (Table 4).

A second approach to analyze curves is by their LDs (Table 5). The maximum LD_{50} difference between two insecticides was 7800-fold, corresponding to *L. botrana* females treated with λ-Cyhalothrin and Thiacloprid. Between species, the maximum LD_{50} difference was 115-fold, corresponding to *L. botrana* and *C. pomonella* females treated with Thiacloprid. Finally, the maximum difference between sexes was 41.5-fold, corresponding to *G. molesta* treated with Chlorpyrifos. Lethal doses LD_{50} and LD_{90} differed in all pairwise comparisons between insecticides, whereas LD_{10} did not (Table 5). Lethal dose comparisons among species were significant in 25 out of 27 pairwise tests. All the exceptions were in the insecticide λ-Cyhalothrin, between *C. pomonella* and *L. botrana* for LD_{50} and between *C. pomonella* and *G. molesta* for LD_{90} (Table 5). Sex differences in LD occurred in 8 out of 9 comparisons in each Chlorpyrifos and Thiacloprid, but were rare in λ-Cyhalothrin (Table 5). Because females of all three species were heavier than males (Table S1) it was expected that females would be less susceptible to the insecticides than males. This prediction was observed in two of the insecticides, λ-Cyhalothrin and Thiacloprid, however in Chlorpyrifos the males of all three species had higher LD_{50} than females, with the notable difference of 41.5-times lower susceptibility for *G. molesta* males than females, as mentioned above (Table 3).

Discussion

Few dose-mortality studies have explored, as we have done in here, the combined effect of insecticides with different modes of action, on adults of several insect species, while simultaneously taking into account the effect of sex. Large mortality differences between insecticides (maximum 7800-fold for LD_{50}) were followed by much lower, yet important, differences between species (115-fold) and sexes (41.5-fold), demonstrating that each of these three factors has a critical effect on adult mortality. Although these factors were not independent from each other, as shown by significant 2nd- to 4th-order model interactions, our results highlight the need to take into account sex as a very significant factor in the context of insecticide and species differences in dose-mortality studies of adult moths.

Insecticide dose-mortality curves of adult Lepidoptera are poorly represented in the scientific literature, probably because most insecticides are mainly designed to kill larval stages, however, neurotoxic insecticides could affect other life stages. Two studies using adult *G. molesta* and *C. pomonella,* and several studies on larva *C. pomonella* have shown, as we do in here, a stronger effect by pyrethroids than by other insecticide types (Linn and Roelofs 1984, Pasquier and Charmillot 2003, Mota-Sánchez *et* al. 2008, Rodríguez *et al*. 2012, Wu *et al*. 2015). Time effect after initial knock-down did not appear to be greatly affected by species or sex, but mostly by insecticide type. The 48h recovery with Thiacloprid (Figure S3), may involve the induction of detoxification enzymes (Terriere 1984), while the increased mortality of Chlorpyrifos at 48h (Figure S3), may be related to the oxidative desulfurization of the P=S group to its corresponding P=O analog by cytochrome P450 monooxygenases, which would increase the toxicity of this insecticide over time (Yu 2008). The recovery observed in the laboratory may not be realized in

the field because moribund insects are probably more susceptible to predation and environmental stress than non-intoxicated individuals.

Species differences in insecticide resistance could be explained either by the activity or quantity of insect degrading enzymes that metabolize the insecticide before it arrives to the target protein, or by mutations at the insecticide´s target site that lower its effect (Nauen and Denholm 2005). Resistance by degrading enzymes should be far more common than mutations at the target site because, at least in the case of neurotoxic insecticides, the target sites are proteins which play fundamental roles in nerve impulse generation and transmission, processes that are fairly conserved across lineages and that should be under strong stabilizing selection and resistant to mutations (Li *et al.* 2007). Changes in activity or quantity of the main detoxifying enzyme types [mixed-function oxidases (MFO), esterases (EST), and glutathione S-transferases (GST)] have been associated with resistance to a large number of insecticides in *C. pomonella* (Reyes *et al.* 2007, 2011; Morales *et al.* 2016). In addition, point mutations in the sodium channel and in the AChE have also been reported in this species as mechanisms of resistance (Reyes *et al.* 2007, Kanga *et al.* 2001). There are comparatively fewer reports of insecticide resistance in *G. molesta* (Glass 1960, Kanga *et* al. 2003, Jones *et al.* 2011), and *L. botrana* (Civolani *et al.* 2014). Lower cuticular penetration of Carbofuran may explain resistance to this insecticide in *G. molesta* (Kanga *et al.* 1997).

For phytophagous insects environmental toxins consist mainly of secondary plant metabolites acquired through ingestion (Li *et al.* 2007), and the function of detoxification enzymes is to make toxins more water soluble (Terriere 1984). It is plausible, then, that species using different food sources may have different detoxifying-enzyme activity levels (Yu 1982), and this may explain why they show different tolerance to insecticides. *C. pomonella's* main agricultural host is apple fruit, *G. molesta´*s is peach shoots and *L. botrana´*s is the flower and fruit of grape vines.

The diversity of host species and host organs consumed by the larvae of these moth species may select for different detoxification mechanisms. For example, *C. pomonella´*s second major agricultural host, walnut fruit, produces high quantities of the naphthoquinone juglone, which is toxic to several insect species but not to the larvae of *C. pomonella* (Piskorski and Dorn 2011). Interestingly, the larva of *G. molesta*, which does not feed on walnuts, is also able to metabolize juglone (Piskorski *et al.* 2011), so ecological factors may not be the only determinants of the quantity and type of detoxification enzymes produced by each species.

One of the most striking findings of our study is the relatively large difference in susceptibility between males and females, and the higher tolerance of males to Chlorpyrifos, in all three species. Higher male tolerance has been reported before in *G. molesta*, where the LC_{50} of females was between 3 and 10 times lower than the LC_{50} of males to three different organophosphate insecticides (Azinphos-methyl, Malathion and Parathion-methyl) (Shearer and Usmani 2001). We confirm the higher male tolerance to organophosphates in *G. molesta*, and in addition we show that higher male tolerance to organophosphates also occurs in the other two tortricids, *C. pomonella* and *L. botrana*. Higher female susceptibility seems to be restricted to organophosphates because, besides our results, *G. molesta* females are less susceptible to carbamates than males (Kanga *et* al. 2001, Shearer and Usmani 2001). The larger LC for females compared to males observed for the neonicotinoid Thiacloprid could be explained by the larger body mass of females, but after correcting by body mass the LD of females was still larger than that of males. This suggests that additional factors, such differences in enzymatic activities and/or quantities, might be playing a role in this case. The sex differences with Chlorpyrifos (females more susceptible) cannot be accounted by body weight, and it is very likely that detoxification enzymes are involved in these differences. de Lame, *et al.* (2001) showed that the larger resistance of male *G. molesta* to three organophosphate insecticides (Paraoxon-methyl, Malaoxon and Diazinon-O-analogue) was the result of sex differences in both, degrading

enzyme activity levels and susceptibility of AChE to the insecticide. Kanga, *et al.* (2001) reported that Ace-1 insensitivity, the major mechanism of carbamate resistance in *G. molesta*, is both sex linked and recessive. Point mutations are probably not involved in sex differences because both sexes share the same chromosomes, except for the W sex chromosome which is only present in females and codifies few gene products (Nguyen *et al.* 2013). A neo-sex chromosome in tortricids emerged from the fusion of the Z chromosome (the other sex chromosome, present in both sexes) and an autosome, and it bears genes encoding for detoxification enzymes (Nguyen *et al.* 2013). It has been suggested that the neo-sex chromosome may be responsible for both, a rapid evolution of this clade and a quick selection response to insecticides (Nguyen *et al.* 2013). Indeed, the expression of AChE genes is larger in males than in females of *C. pomonella* and *L. botrana* (Nguyen *et al.* 2013). It remains to be tested if the neo-sex chromosome is also responsible for the differential sex response of the three tortricid species to insecticides.

The results of our study have practical implications. First, our dose-mortality curves for susceptible strains provide a diagnostic baseline to test possible resistance cases in field populations using adult insects, as in other susceptibility-resistance studies that use larvae or adult insects (Pasquier and Charmillot 2003, Reyes *et al.* 2007, Jones *et al.* 2011, Wu *et al.* 2015). Second, resistance is expressed in both adults and larvae (Varela *et al.* 1993), but the use of adult instead of larvae in this kind of studies is advantageous because of the easier, faster and cheaper procedure with adults than with larvae (Kanga *et al.* 1997). For example, adult individuals can be easily obtained in the field with monitoring traps (Bosch *et al.* 2016). Third, our dose-mortality results help estimate sublethal doses which could affect the behavior and physiology of these insects (Haynes 1988). Finally, differential sex response to insecticides should be considered in integrated pest management programs. Shearer and Usmani (2001)

indicate that male pheromone trap catches may be unfit to monitor threshold population levels if males are less susceptible than females to insecticide.

Acknowledgments

MAN-R was supported by a Ph.D. fellowship from the University of Lleida. This study was supported by research Grant AGL2013-49164-C2-1 MINECO, Spain.

References Cited

Beers, E.H., J.F. Brunner, J.E. Dunley, M. Doerr, and K. Granger. 2005. Role of neonicotinyl insecticides in Washington apple integrated pest management. Part II. Nontarget effects on integrated mite control. J. Insect. Sci. 5(1):16.

Bosch, D., M.A. Rodríguez, and J. Avilla. 2016. Captures of MFO-resistant *Cydia pomonella* adults as affected by lure, crop management system and flight. B. Entomol. Res. 106(1):54-62.

Brunner, J.F., E.H. Beers, J.E. Dunley, M. Doerr, and K. Granger. 2005. Role of neonicotinyl insecticides in Washington apple integrated pest management. Part I. Control of lepidopteran pests. J. Insect. Sci. 5(1):14.

Casida, J.E. 2009. Pest toxicology: the primary mechanisms of pesticide action. Chem. Res. Toxicol. 22(4):609-619.

Cichón, L.B., J. Soleno, O.L. Anguiano, S.A.S. Garrido, and C.M. Montagna. 2013. Evaluation of cytochrome P450 activity in field populations of *Cydia pomonella*

(Lepidoptera: Tortricidae) resistant to azinphosmethyl, acetamiprid, and thiacloprid. J. Econ. Entomol. 106:939-944.

Cutler, G.C. 2013. Insects, insecticides and hormesis: evidence and considerations for study. Dose-Response. 11:154–177.

Civolani, S., M. Boselli, A. Butturini, M. Chicca, E.A. Fano, and S. Cassanelli. 2014. Assessment of Insecticide Resistance of *Lobesia botrana* (Lepidoptera: Tortricidae) in Emilia-Romagna Region. J. Econ. Entomol. 107:1245-1249.

Damos, P., L.A.E. Colomar, and C. Ioriatti. 2015. Integrated fruit production and pest management in Europe: the apple case study and how far we are from the original concept? Insects. 6(3):626-657.

de Lame, F.M., J.J. Hong, P.W. Shearer, and L.B. Brattsten. 2001. Sex-related differences in the tolerance of Oriental fruit moth (*Grapholita molesta*) to organophosphate insecticides. Pest Manag. Sci. 57(9):827-832.

Fernandes, M.E., F.M. Alves, R.C. Pereira, L.A. Aquino, F.L. Fernandes, and J.C. Zanuncio. 2016. Lethal and sublethal effects of seven insecticides on three beneficial insects in laboratory assays and field trials. Chemosphere. 156:45-55.

Glass, E.H. 1960. Current status of pesticide resistance in insects and mites attaching deciduous orchard crops. Misc. Pub. Entomol. Soc. Am. 2:17-25.

Grigg-McGuffin, K., I.M. Scott, S. Bellerose, G. Chouinard, D. Cormier, and C. Scott-Dupree. 2015. Susceptibility in field populations of codling moth, *Cydia pomonella* (L.)(Lepidoptera: Tortricidae), in Ontario and Quebec apple orchards to a selection of insecticides. Pest Manag. Sci. 71(2):234-242.

Guedes, R.N.C., G. Smagghe, J.D. Stark, and N. Desneux. 2015. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu. Rev. Entomol. 61:43-62.

Guillette, L.J., and T. Iguchi. 2012. Life in a contaminated world. Science. 337(6102):1614-1615.

Haynes, K.F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Annu. Rev. Entomol. 33(1):149-168.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical J. 50(3):346-363.

Ioriatti, C., G. Anfora, G. Angeli, S. Civolani, S. Schmidt, and E. Pasqualini. 2009a. Toxicity of emamectin benzoate to *Cydia pomonella* (L.) and *Cydia molesta* (Busck) (Lepidoptera: Tortricidae): laboratory and field tests. Pest Manag. Sci. 65(3):306-312.

Ioriatti, C., G. Anfora, G. Angeli, V. Mazzoni, and F. Trona. 2009b. Effects of chlorantraniliprole on eggs and larvae of *Lobesia botrana* (Denis & Schiffermüller)(Lepidoptera: Tortricidae). Pest Manag. Sci. 65(6), 717-722.

Ioriatti, C., G. Anfora, M. Tasin, A. de Cristofaro, P. Witzgall, and A. Lucchi. 2011. Chemical ecology and management of *Lobesia botrana* (Lepidoptera: Tortricidae). J. Econ. Entomol. 104(4):1125-1137.

(IRAC) Insecticide Resistance Action Committee. 2016. Resistance - IRAC. http://www.irac-online.org/about/resistance/. Accessed 12 February 2016.

Ivaldi-Sender, C. 1974. Techniques simples pour elevage permanent de la tordeuse orientale, *Grapholita molesta* (Lep., Tortricidae), sur milieu artificiel. Ann. Zoolog. Ecol. Anim. 6:337–343.

Jones, M.M., JL Robertson, and RA Weinzierl. 2011. Susceptibility of oriental fruit moth (Lepidoptera: Tortricidae) to two pyrethroids and a proposed diagnostic dose of esfenvalerate for field detection of resistance. J. Econ. Entomol. 104(3):1031-1037.

Kanga, L.H.B., D.J. Pree, F.W. Plapp, and J.L. Van Lier. 2001. Sex-linked altered acetylcholinesterase resistance to carbamate insecticides in adults of the oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae). Pestic. Biochem. Phys. 71(1):29-39.

Kanga, L.H., D.J. Pree, J.L. Van Lier, and G.M. Walker. 2003. Management of insecticide resistance in oriental fruit moth (*Grapholita molesta*; Lepidoptera: Tortricidae) populations from Ontario. Pest Manag. Sci. 59(8):921-927.

Kanga, L.H.B., D.J. Pree, J.L. Van Lier, and K.J. Whitty. 1997. Mechanisms of resistance to organophosphorus and carbamate insecticides in Oriental fruit moth populations (*Grapholita molesta* Busck). Pestic. Biochem. Phys. 59(1):11-23.

Kirk, H., S. Dorn, and D. Mazzi. 2013. Worldwide population genetic structure of the oriental fruit moth (*Grapholita molesta*), a globally invasive pest. BMC Ecology. 13(1):12.

Knight, A.L. 2000. Tebufenozide targeted against codling moth (Lepidoptera: Tortricidae) adults, eggs, and larvae. J. Econ. Entomol. 93(6):1760-1767.

Li, X., M.A. Schuler, and M.R. Berenbaum. 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol. 52:231-253.

Linn, C.E., and W.L. Roelofs. 1984. Sublethal effects of neuroactive compounds on pheromone response thresholds in male oriental fruit moths. Arch. Insect. Biochem. 1(4):331-344.

(MAGRAMA). Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente. 2015. Registro de Productos Fitosanitarios.

http://www.magrama.gob.es/es/agricultura/temas/sanidad-vegetal/productos-fitosanitarios/registro/menu.asp. Accessed 09 June 2015.

Magalhaes, L. C., and J.F. Walgenbach. 2011. Life stage toxicity and residual activity of insecticides to codling moth and oriental fruit moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 104(6), 1950-1959.

Morales, L.B.P., R.A. Alzogaray, L. Cichón, S. Garrido, J. Soleño, and C.M. Montagna. 2016. Effects of chlorpyrifos on enzymatic systems of *Cydia pomonella* (Lepidoptera: Tortricidae) adults. Insect Sci. DOI 10.1111/1744-7917.12307.

Mota-Sanchez, D., J.C. Wise, R.V. Poppen, L.J. Gut, and R.M. Hollingworth. 2008. Resistance of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), larvae in Michigan to insecticides with different modes of action and the impact on field residual activity. Pest Manag. Sci. 64(9):881-890.

Nauen, R., and I. Denholm. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. Arch. Insect. Biochem. 58(4):200-215.

Nayak, M.K., and G.J. Daglish. 2006. Potential of imidacloprid to control four species of psocids (Psocoptera: Liposcelididae) infesting stored grain. Pest Manag. Sci. 62(7):646-650.

Nguyen, P., M. Sýkorová, J. Šíchová, V. Kůta, M. Dalíková, R.Č. Frydrychová, L.G. Neven, K. Sahara, and F. Marec. 2013. Neo-sex chromosomes and adaptive potential in tortricid pests. P. Natl. Acad. Sci. USA. 110(17):6931-6936.

Pasquier, D., and P.J. Charmillot. 2003. Effectiveness of twelve insecticides applied topically to diapausing larvae of the codling moth, *Cydia pomonella* L. Pest Manag. Sci. 60(3): 305-308.

Pavan, F., E. Cargnus, G. Bigot, and P. Zandigiacomo. 2014. Residual activity of insecticides applied against *Lobesia botrana* and its influence on resistance management strategies. B. Insectol. 67(2):273-280.

Piskorski, R., and S. Dorn. 2011. How the oligophage codling moth *Cydia pomonella* survives on walnut despite its secondary metabolite juglone. J. Insect. Physiol. 57(6):744-750.

Piskorski, R., S. Ineichen, and S. Dorn. 2011. Ability of the oriental fruit moth *Grapholita molesta* (Lepidoptera: Tortricidae) to detoxify juglone, the main secondary metabolite of the non-host plant walnut. J. Chem. Ecol. 37(10):1110-1116.

Preisler, H.K., and J.L. Robertson. 1989. Analysis of time-dose-mortality data. J. Econ. Entomol. 82(6):1534-1542.

(R) R Core Team. 2016. R: A language and environment for statistical computing. http://www.R-project.org/. Accessed 27 July 2016.

Reyes, M., B. Collange, M. Rault, S. Casanelli, and B. Sauphanor. 2011. Combined detoxification mechanisms and target mutation fail to confer a high level of resistance to organophosphates in *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Pestic. Biochem. Phys. 99(1):25-32.

Reyes, M., P. Franck, P.J. Charmillot, C. Ioriatti, J. Olivares, E. Pasqualini, and B. Sauphanor. 2007. Diversity of insecticide resistance mechanisms and spectrum in European populations of the codling moth, *Cydia pomonella*. Pest Manag. Sci. 63(9):890-902.

Reyes, M., and B. Sauphanor. 2008. Resistance monitoring in codling moth: a need for standardization. Pest Manag. Sci. 64(9), 945-953.

Rodríguez, M.A., D. Bosch, and J. Avilla. 2012. Azinphos-methyl and carbaryl resistance in adults of the codling moth (*Cydia pomonella* (L.), Lepidoptera: Tortricidae) from Northeastern Spain. Pestic. Biochem. Phys. 103(1):43-48.

Rodríguez, M.A., T. Marques, D. Bosch, and J. Avilla. 2011. Assessment of insecticide resistance in eggs and neonate larvae of *Cydia pomonella* (Lepidoptera: Tortricidae). Pestic. Biochem. Phys. 100(2):151-159.

Rodriguez-Saona, C., A.C. Wanumen, J. Salamanca, R. Holdcraft, and V. Kyryczenko-Roth. 2016. Toxicity of insecticides on various life stages of two tortricid pests of cranberries and on a non-target predator. Insects. 7(2):15.

Sáenz-de-Cabezón Irigaray, F.J., V. Marco, F.G. Zalom, and I. Pérez-Moreno. 2005. Effects of methoxyfenozide on *Lobesia botrana* Den & Schiff (Lepidoptera: Tortricidae) egg, larval and adult stages. Pest Manag. Sci. 61(11):1133-1137.

Shearer, P.W., and K.A. Usmani. 2001. Sex-related response to organophosphorus and carbamate insecticides in adult Oriental fruit moth, *Grapholita molesta*. Pest Manag. Sci. 57(9):822-826.

Siegwart, M., L.B. Monteiro, S. Maugin, J. Olivares, S.M. Carvalho, and B. Sauphanor. 2011. Tools for resistance monitoring in oriental fruit moth (Lepidoptera: Tortricidae) and first assessment in Brazilian populations. J. Econ. Entomol. 104(2):636-645.

Sparks, T.C., and R. Nauen. 2015. IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Phys. 121:122-128.

Terriere, L.C. 1984. Induction of detoxication enzymes in insects. Annu. Rev. Entomol. 29(1):71-88.

Vandekerkhove, B., and P. de Clercq. 2004. Effects of an encapsulated formulation of lambda-cyhalothrin on *Nezara viridula* and its predator *Podisus maculiventris* (Heteroptera: Pentatomidae). Fla. Entomol. 87(2):112-118.

Varela, L.G., S.C. Welter, V.P. Jones, J.F. Brunner, and H. Riedl. 1993. Monitoring and characterization of insecticide resistance Codling moth (Lepidoptera: Tortricidae) in four Western States. J. Econ. Entomol. 86(1):1-10.

Venables, W.N., and B.D. Ripley. 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.

Wu, Z.W., Y.L. Zhang, and S.Q. Shang. 2015. Effectiveness of 12 insecticides to a laboratory population of *Cydia pomonella* (Lepidoptera: Tortricidae) newly established in China. J. Econ. Entomol. 108(3):1271-1278.

Yang, X., X. Li, and Y. Zhang. 2013. Molecular cloning and expression of CYP9A61: a chlorpyrifos-ethyl and lambda-cyhalothrin-inducible cytochrome P450 cDNA from *Cydia pomonella*. Int. J. Mol. Sci. 14(12):24211-24229.

Yu, S. J. 1982. Host plant induction of glutathione S-transferase in the fall armyworm. Pestic. Biochem. Phys. 18(1):101-106.

Yu, S.J. 2008. The Toxicology and Biochemistry of Insecticides. CRC. Press, Boca Raton, FL.

Zotti, M.J., A.D. Grutzmacher, I.H. Lopes, and G. Smagghe. 2013. Comparative effects of insecticides with different mechanisms of action on *Chrysoperla externa* (Neuroptera: Chrysopidae): Lethal, sublethal and dose–response effects. Insect. Sci. 20(6):743-752.

Figure 1. Effect of insecticide type (λ-Cyhalothrin, Chlorpyrifos, and Thiacloprid) and dose (ng of insecticide per gram of insect dry weight; in logarithmic scale) on the proportion of moribund and dead adult males and females of *Cydia pomonella*, *Grapholita molesta*, and *Lobesia botrana* 24 h after treatment. The symbols indicate the observed values (N=60-116), while the curves are the estimated values from probit regression.

Figure 1.

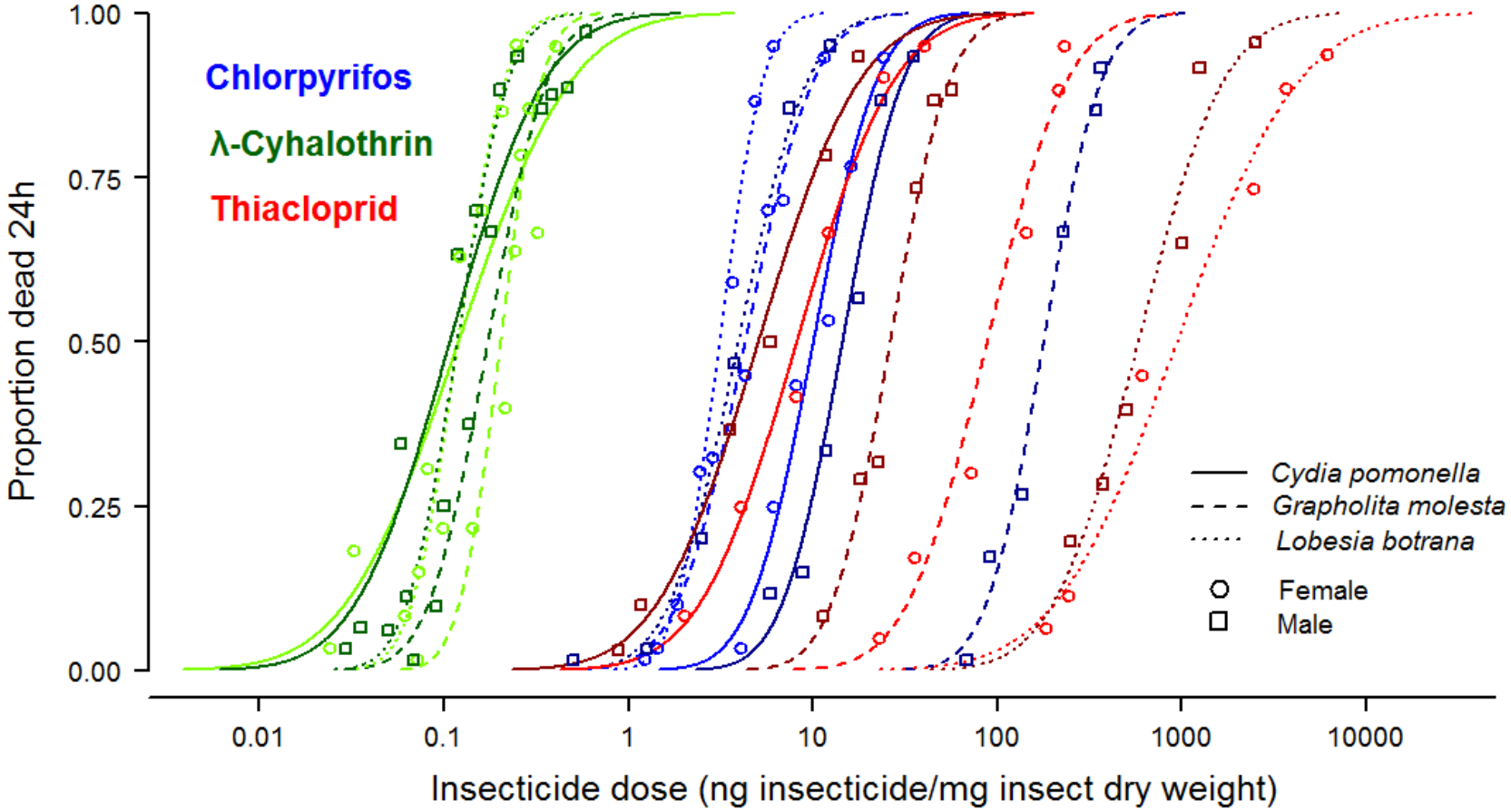
Chlorpyrifos
λ-Cyhalothrin
Thiacloprid
Proportion dead 24h
1.00
0.75
0.50
0.25
0.00
0.01
0.1
1
10
100
1000
10000
Insecticide dose (ng insecticide/mg insect dry weight)
Cydia pomonella
Grapholita molesta
Lobesia botrana
Female
Male

Table 1. Model comparison for the analysis of percent mortality at 24h as a function of insecticide dose. Models with increasing numbers of parameter interactions (insecticide type, dose, species and sex) were compared pairwise using the likelihood ratio test (LRT) and Akaike Information Criterion (AIC).

Model type	**Number of parameters**	**AIC[a]**	**LRT p-value**
Null	1	4000.5	-
Main effects	7	3088.2	<0.00001
Main effects and 2nd-order interactions	20	1249.5	<0.00001
Main effects and 2nd and 3rd-order interactions	32	623.04	<0.00001
Main effects and 2nd, 3rd and 4th-order interactions	36	600.13	<0.00001

[a] Models with lower AIC indicate a better model fit

Table 2. Analysis of deviance table for the model with all main effects and second, third and fourth-order interactions. Main effects are: insecticide type (Chlorpyrifos, λ-Cyhalothrin and Thiacloprid), moth species (*C. pomonella*, *G. molesta* and *L. botrana*), sex and insecticide dose (ng of insecticide per mg of insect dry weight).

Model terms	**Df** [a]	**Deviance**	**Resid. Df** [b]	**Resid. Dev** [c]	**Pr (>Chi)**
NULL			107	3588.7	
species	2	2.32	105	3586.3	0.3127
sex	1	1.40	104	3584.9	0.2360
insecticide	2	7.84	102	3577.1	0.0198
dose	1	912.81	101	2664.3	<0.0001
species:sex	2	19.62	99	2644.7	<0.0001
species:insecticide	4	959.85	95	1684.8	<0.0001
species:dose	2	483.91	93	1200.9	<0.0001
sex:insecticide	2	344.06	91	856.8	<0.0001
sex:dose	1	2.27	90	854.6	0.1321
insecticide:dose	2	54.96	88	799.6	<0.0001
species:sex:insecticide	4	568.98	84	230.6	<0.0001
species:sex:dose	2	0.27	82	230.4	0.8744
species:insecticide:dose	4	73.77	78	156.6	<0.0001
sex:insecticide:dose	2	7.43	76	149.2	0.0244
species:sex:insecticide:dose	4	30.92	72	118.2	<0.0001

[a] Degrees of freedom
[b] Residual degrees of freedom
[c] Residual deviance

Table 3. Estimated intercepts and slopes of the probit regression models, and lethal doses LD_{10}, LD_{50} and LD_{90}, with their standard errors and 95% confident intervals. Intercept and slope are "dimensionless" model parameter whereas LDs are in ng of insecticide per mg of adult insect dry weight. Estimations are provided by groups: for each insecticide (Chlorpyrifos, λ-Cyhalothrin and Thiacloprid; independent of sex and species; top section), for each species (*C. pomonella*, *G. molesta* and *L. botrana*; within insecticide and independent of sex, middle section) and for each sex (within species and insecticide; bottom section). Heterogeneity factor (HF) indicates curve fit.

Insecticide	Species	Sex	n	Intercept		Slope		LD_{10}		LD_{50}		LD_{90}		HF[a]
				beta (SE)	(95%CI)	beta (SE)	(95%CI)	Dose	(95%CI)	Dose	(95%CI)	Dose	(95%CI)	
Chlorpyrifos			2234	-0.63 (0.05)	(-0.72; -0.53)	0.58 (0.04)	(0.49; 0.66)	0.07	(0.04; 0.15)	12.30	(9.89; 15.28)	$2.09 \cdot 10^{+3}$	$(937.64; 4.66 \cdot 10^{+3})$	26.1
λ-Cyhalothrin			2364	2.40 (0.09)	(2.23; 2.59)	2.82 (0.11)	(2.63; 3.03)	0.05	(0.05; 0.05)	0.14	(0.13; 0.15)	0.40	(0.37; 0.44)	7.8
Thiacloprid			2204	-0.72 (0.06)	(-0.83; -0.60)	0.42 (0.03)	(0.37; 0.48)	0.05	(0.02; 0.13)	49.09	(36.54; 65.96)	$5.20 \cdot 10^{+4}$	$(2.03 \cdot 10^{+4}; 1.33 \cdot 10^{+5})$	23.4
Chlorpyrifos	***C. pomonella***		720	-3.72 (0.26)	(-4.23; -3.23)	3.44 (0.23)	(3.00; 3.90)	5.12	(4.49; 5.85)	12.09	(11.27; 12.98)	28.55	(24.96; 32.66)	4.0
	G. molesta		744	-0.37 (0.09)	(-0.56; -0.19)	0.25 (0.06)	(0.14; 0.36)	$2.5 \cdot 10^{-4}$	$(1.5 \cdot 10^{-6}; 4.1 \cdot 10^{-2})$	30.28	(13.20; 69.48)	$3.69 \cdot 10^{+6}$	$(2.09 \cdot 10^{+4}; 6.49 \cdot 10^{+8})$	29.4
	L. botrana		770	-2.14 (0.14)	(-2.42; -1.87)	3.95 (0.25)	(3.49; 4.44)	1.65	(1.47; 1.84)	3.47	(3.25; 3.71)	7.33	(6.52; 8.24)	6.3
λ-Cyhalothrin	***C. pomonella***		736	2.13 (0.14)	(1.86; 2.41)	2.26 (0.13)	(2.01; 2.52)	0.03	(0.03; 0.04)	0.11	(0.10; 0.13)	0.42	(0.35; 0.51)	3.6
	G. molesta		862	3.09 (0.19)	(2.72; 3.47)	4.24 (0.26)	(3.73; 4.77)	0.09	(0.08; 0.10)	0.19	(0.18; 0.20)	0.37	(0.34; 0.41)	3.2
	L. botrana		766	4.41 (0.26)	(3.91; 4.93)	4.77 (0.27)	(4.25; 5.31)	0.06	(0.06; 0.07)	0.12	(0.11; 0.13)	0.22	(0.20; 0.24)	1.3
Thiacloprid	***C. pomonella***		725	-1.78 (0.13)	(-2.04; -1.53)	2.19 (0.14)	(1.92; 2.48)	1.68	(1.36; 2.07)	6.45	(5.75; 7.24)	24.77	(20.26; 30.28)	2.2
	G. molesta		733	-3.02 (0.24)	(-3.50; -2.55)	1.82 (0.14)	(1.55; 2.11)	9.01	(6.84; 11.86)	45.48	(40.12; 51.56)	229.63	(173.68; 303.60)	15.8
	L. botrana		746	-5.86 (0.38)	(-6.61; -5.13)	2.06 (0.13)	(1.81; 2.32)	167.34	(134.40; 208.37)	702.34	(623.71; 790.89)	$2.95 \cdot 10^{+3}$	$(2.39 \cdot 10^{+3}; 3.64 \cdot 10^{+3})$	3.6
Chlorpyrifos	***C. pomonella***	**Female**	360	-3.70 (0.35)	(-4.42; -3.03)	3.69 (0.34)	(3.03; 4.39)	4.53	(3.82; 5.38)	10.08	(9.18; 11.08)	22.43	(18.85; 26.70)	1.3
		Male	360	-4.50 (0.42)	(-5.32; -3.72)	3.88 (0.35)	(3.21; 4.58)	6.77	(5.75; 7.97)	14.50	(13.24; 15.88)	31.05	(26.37; 36.56)	1.7
	G. molesta	**Female**	368	-2.17 (0.23)	(-2.65; -1.73)	3.43 (0.33)	(2.79; 4.11)	1.82	(1.49; 2.23)	4.31	(3.90; 4.77)	10.20	(8.51; 12.23)	0.7
		Male	376	-9.13 (0.74)	(-10.62; -7.71)	4.05 (0.33)	(3.43; 4.71)	86.29	(74.70; 99.69)	178.77	(163.55; 195.42)	370.36	(319.86; 428.84)	1.2
	L. botrana	**Female**	387	-2.67 (0.23)	(-3.14; -2.24)	5.39 (0.44)	(4.56; 6.28)	1.81	(1.62; 2.02)	3.13	(2.92; 3.35)	5.41	(4.83; 6.05)	0.3
		Male	383	-2.02 (0.20)	(-2.42; -1.65)	3.38 (0.30)	(2.83; 3.98)	1.65	(1.36; 2.01)	3.96	(3.53; 4.44)	9.48	(7.88; 11.40)	3.4
λ-Cyhalothrin	***C. pomonella***	**Female**	362	1.91 (0.19)	(1.54; 2.28)	2.07 (0.18)	(1.72; 2.44)	0.03	(0.02; 0.04)	0.12	(0.10; 0.14)	0.50	(0.37; 0.68)	4.8
		Male	374	2.39 (0.22)	(1.98; 2.84)	2.47 (0.20)	(2.10; 2.87)	0.03	(0.03; 0.04)	0.11	(0.09; 0.13)	0.36	(0.27; 0.46)	2.9
	G. molesta	**Female**	459	3.93 (0.41)	(3.18; 4.75)	5.67 (0.63)	(4.54; 6.94)	0.12	(0.10; 0.14)	0.20	(0.19; 0.22)	0.34	(0.31; 0.38)	2.8
		Male	403	3.00 (0.26)	(2.51; 3.50)	3.94 (0.32)	(3.33; 4.58)	0.08	(0.07; 0.10)	0.17	(0.16; 0.19)	0.37	(0.32; 0.43)	2.3
	L. botrana	**Female**	364	4.62 (0.41)	(3.85; 5.44)	5.01 (0.42)	(4.21; 5.87)	0.07	(0.06; 0.08)	0.12	(0.11; 0.13)	0.22	(0.19; 0.25)	1.9
		Male	402	4.26 (0.34)	(3.61; 4.94)	4.60 (0.35)	(3.93; 5.30)	0.06	(0.06; 0.07)	0.12	(0.11; 0.13)	0.23	(0.20; 0.26)	1.3
Thiacloprid	***C. pomonella***	**Female**	361	-2.20 (0.22)	(-2.64; -1.78)	2.41 (0.22)	(2.00; 2.85)	2.40	(1.83; 3.16)	8.18	(7.05; 9.50)	27.82	(21.71; 35.65)	0.6
		Male	364	-1.63 (0.16)	(-1.95; -1.32)	2.31 (0.20)	(1.93; 2.71)	1.41	(1.08; 1.84)	5.07	(4.33; 5.93)	18.19	(13.89; 23.84)	0.7
	G. molesta	**Female**	371	-5.71 (0.48)	(-6.68; -4.80)	2.94 (0.24)	(2.48; 3.42)	32.28	(26.16; 39.84)	88.17	(77.91; 99.77)	240.81	(198.04; 292.83)	1.8
		Male	362	-5.64 (0.53)	(-6.69; -4.63)	3.97 (0.36)	(3.28; 4.69)	12.52	(10.60; 14.79)	26.35	(24.14; 28.77)	55.45	(47.65; 64.53)	0.8
	L. botrana	**Female**	369	-5.69 (0.46)	(-6.63; -4.80)	1.91 (0.15)	(1.62; 2.22)	201.42	(147.00; 276.00)	941.34	$(776.78; 1.14 \cdot 10^{+3})$	$4.40 \cdot 10^{+3}$	$(3.27 \cdot 10^{+3}; 5.93 \cdot 10^{+3})$	1.0
		Male	377	-7.58 (0.73)	(-9.03; -6.19)	2.74 (0.26)	(2.25; 3.26)	198.13	(154.86; 253.50)	581.53	(513.58; 658.47)	$1.71 \cdot 10^{+3}$	$(1.37 \cdot 10^{+3}; 2{,}13 \cdot 10^{+3})$	1.9

[a] Heterogeneity factor = χ^2/dF

Table 4. Pairwise comparison of intercepts and slopes between a) insecticides (Chlorpyrifos, λ-Cyhalothrin and Thiacloprid; independent of species and sex; top section), b) species (*C. pomonella*, *G. molesta* and *L. botrana*; within insecticide and independent of sex; middle section), and c) sex (within species and insecticide; bottom section). The numbers represent the difference between each pair of estimated values, and are followed in brackets by the p-values of these differences (Tukey test, $p<0.05$, after GLM).

Insecticide	**Species**	**Sex differences**		**Species differences**				**Insecticide differences**			
				C. pomonella		***G. molesta***		**Chlorpyrifos**		**λ-Cyhalothrin**	
		Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
Chlorpyrifos	***C. pomonella***	0.80 (0.14)	-0.19 (0.71)	--	--	--	--	--	--	--	--
	G. molesta	6.95 (<0.0001)	-0.63 (0.18)	-3.35 (<0.0001)	3.18 (<0.0001)	--	--				
	L. botrana	-0.65 (0.03)	2.02 (<0.0001)	-1.58 (<0.0001)	-0.52 (0.13)	1.76 (<0.0001)	-3.70 (<0.0001)				
λ-Cyhalothrin	***C. pomonella***	-0.49 (0.10)	-0.41 (0.14)	--	--	--	--	-3.03 (<0.0001)	-2.25 (<0.0001)	--	--
	G. molesta	0.93 (0.05)	1.73 (0.01)	-0.96 (<0.0001)	-1.98 (<0.0001)	--	--				
	L. botrana	0.37 (0.49)	0.42 (0.45)	-2.28 (<0.0001)	-2.51 (<0.0001)	-1.32 (<0.0001)	-0.53 (0.16)				
Thiacloprid	***C. pomonella***	-0.57 (0.04)	0.10 (0.73)	--	--	--	--	0.09 (0.24)	0.15 (0.003)	3.12 (<0.0001)	2.40 (<0.0001)
	G. molesta	-0.08 (0.91)	-1.03 (0.02)	1.25 (<0.0001)	0.37 (0.07)	--	--				
	L. botrana	1.89 (0.03)	-0.83 (0.006)	4.08 (<0.0001)	0.14 (0.48)	2.84 (<0.0001)	-0.24 (0.22)				

Table 5. Pairwise comparison of lethal doses LD_{10}, LD_{50} and LD_{90} between a) insecticides (Chlorpyrifos, λ-Cyhalothrin and Thiacloprid; independent of species and sex; top section), b) species (*C. pomonella*, *G. molesta* and *L. botrana*; within insecticide and independent of sex; middle section), and c) sex (within species and insecticide; bottom section). Numbers are the differences of the estimated values, and in brackets the p-values of these differences (Z-score, $p<0.05$, after GLM).

Insecticide	**Species**	**Sex differences**			**Species differences**						**Insecticide differences**					
					C. pomonella			***G. molesta***			**Chlorpyrifos**			**λ-Cyhalothrin**		
		LD_{10}	LD_{50}	LD_{90}	LD_{10}	LD_{50}	LD_{90}	LD_{10}	LD_{50}	LD_{90}	LD_{10}	LD_{50}	LD_{90}	LD_{10}	LD_{50}	LD_{90}
Chlorpyrifos	***C. pomonella***	-2.24 (0.00)	-4.42 (0.00)	-8.62 (0.01)	--	--	--	--	--	--	--	--	--	--	--	--
	G. molesta	-84.47 (0.00)	-174.46 (0.00)	-360.16 (0.00)	5.12 (0.00)	-18.19 (0.03)	$-3.69\cdot10^{+3}$ (0.00)	--	--	--						
	L. botrana	0.16 (0.42)	-0.83 (0.00)	-4.07 (0.00)	3.48 (0.00)	8.62 (0.00)	21.22 (0.00)	-1.65 (0.00)	26.81 (0.00)	$3.69\cdot10^{+3}$ (0.00)						
λ-Cyhalothrin	***C. pomonella***	-0.00 (0.50)	0.01 (0.37)	0.14 (0.10)	--	--	--	--	--	--	0.02 (0.31)	12.16 (0.00)	$2.09\cdot10^{+3}$ (0.00)	--	--	--
	G. molesta	0.04 (0.00)	0.03 (0.00)	0.03 (0.43)	-0.06 (0.00)	-0.07 (0.00)	0.05 (0.27)	--	--	--						
	L. botrana	0.00 (0.47)	0.00 (0.88)	-0.01 (0.62)	-0.03 (0.00)	-0.00 (0.52)	0.20 (0.00)	0.03 (0.00)	0.07 (0.00)	0.15 (0.00)						
Thiacloprid	***C. pomonella***	0.99 (0.01)	3.11 (0.00)	9.63 (0.02)	--	--	--	--	--	--	0.03 (0.48)	-36.79 (0.00)	$-4.99\cdot10^{+4}$ (0.00)	0.00 (0.90)	48.95 (0.00)	$-5.20\cdot10^{+4}$ (0.00)
	G. molesta	19.76 (0.00)	61.82 (0.00)	185.37 (0.00)	-7.33 (0.00)	-39.03 (0.00)	-204.86 (0.00)	--	--	--						
	L. botrana	3.28 (0.94)	359.81 (0.00)	$2.69\cdot10^{+3}$ (0.00)	-165.66 (0.00)	-695.89 (0.00)	$-2.92\cdot10^{+3}$ (0.00)	-158.33 (0.00)	-656.86 (0.00)	$-2.72\cdot10^{+3}$ (0.00)						

Comparative effect of three neurotoxic insecticides with different modes of action on adult males and females of three tortricid moth pests

Miguel A. Navarro-Roldán[1], Jesús Avilla[2], Dolors Bosch[3], Joan Valls[4] and César Gemeno[1].

[1] Department of Crop and Forest Sciences. University of Lleida (UdL). 25198-Lleida, Spain

[2] Department of Crop and Forest Sciences. Agrotecnio. University of Lleida (UdL). 25198-Lleida, Spain

[3] Department of Sustainable Crop Protection. Food and Agriculture Research Institute (IRTA) 25198-Lleida, Spain

[4] Biostatistics Unit. Biomedical Research Institute (IRBLLEIDA). 25198-Lleida, Spain

Supplementary material.

Table S1. Differences in adult body dry weight by species and sex. Means followed by different letters are significantly different (Tukey test, $p<0.05$, after ANOVA).

Species	Sex	Mean	±	SEM (mg)	
C. pomonella	**Female**	12.41	±	0.63	a
	Male	8.47	±	0.34	b
G. molesta	**Female**	3.54	±	0.11	c
	Male	2.18	±	0.08	d
L. botrana	**Female**	4.09	±	0.10	c
	Male	1.98	±	0.04	d

Figure S1. Comparison of insecticide concentration (in ng; coloured curves) and dose (in ng of insecticide/mg of insect dry weight; grey colour curves, same as in Fig. 1 of the main text) for the 24h mortality of the three insecticides on the three moth species. Colours and symbols as shown in Fig. 1 of the main text, but in the present figure the x-axis is absolute insecticide concentration, instead of dose. As predicted (Figure S2), dose-response curves are displaced to the left from their homologous concentration-response curves, and the displacement is stronger for heavier insects (e.g., *C. pomonella* and the females).

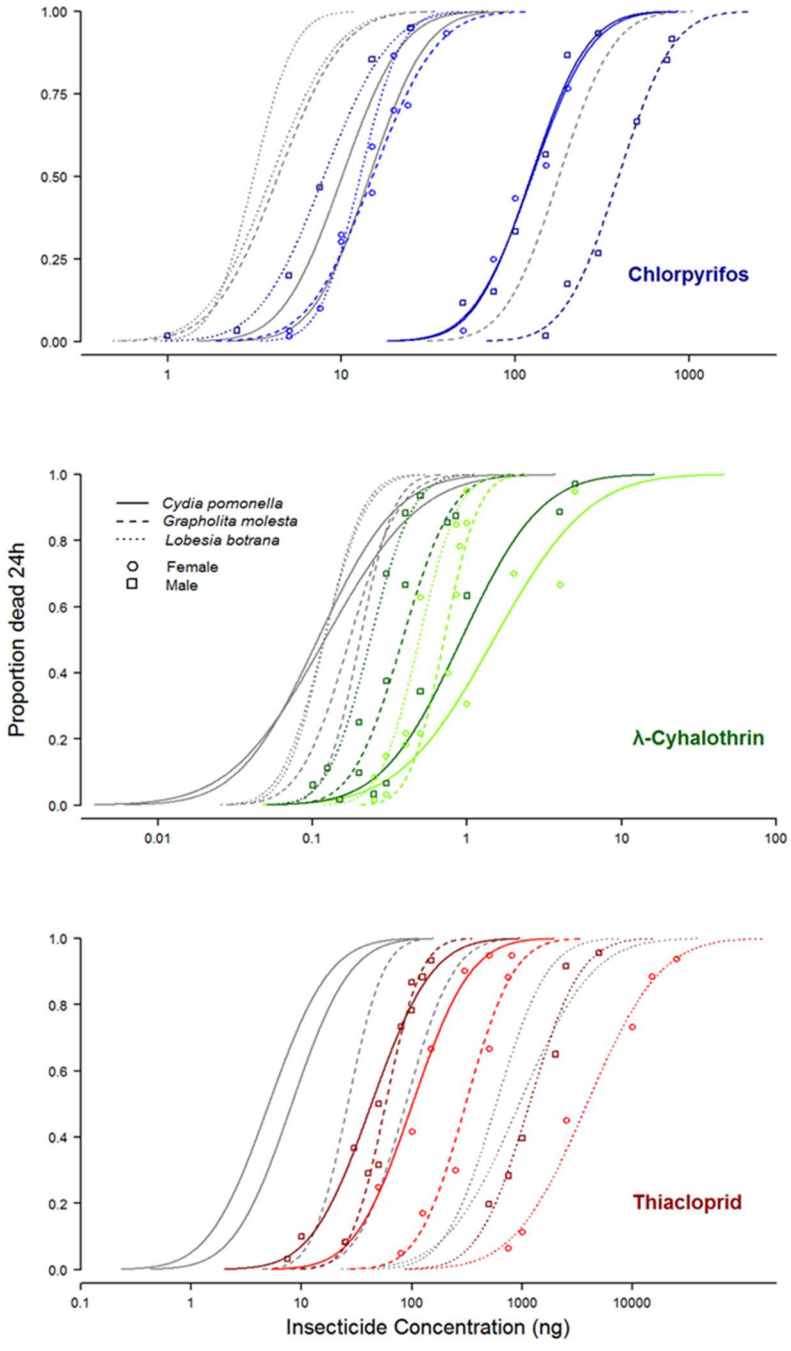

Figure S2. Theoretical relative position of mortality curves estimated with the absolute insecticide concentration (top chart) or the quantity of insecticide per mass unit (i.e., dose; bottom chart). The larger animal, *Cydia pomonella*, is represented with a continuous curve and the smaller animal, *Lobesia botrana*, is represented with a dashed curve. In case "A" the larger species is as susceptible as the smaller species in the concentration scale (top), which occurs if the larger species is more susceptible than the smaller species per unit of mass, as is shown in the dose scale on the bottom. In case "B", the large species is more susceptible than the smaller species in the concentration scale, and, correspondingly, in the dose scale this difference should be larger. In cases "C" and "D" the smaller species is more susceptible than the larger species in the concentration scale, which indicates that the smaller species is either as sensitive as, or less sensitive than the larger species per unit of mass, as shown in the dose scale.

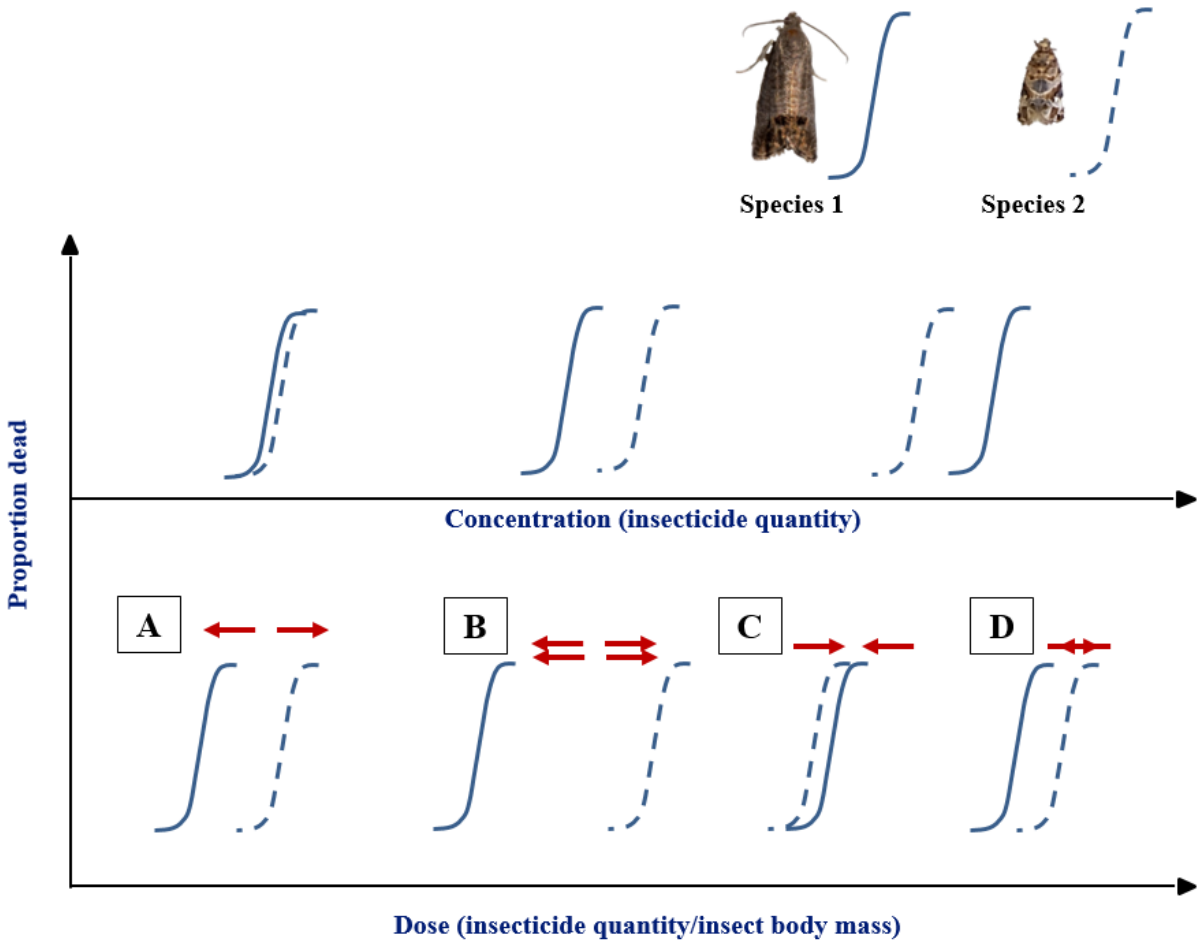

Figure S3. Effect of time after insecticide application on the proportion of mortality (dead + moribund). Grey curves represent mortality at 24 h (as shown in full detail in Fig. 1 of the main text). Colour curves represent mortality at 48 h. For Thiacloprid the curves experimented a displacement to the right in the dose axis, which indicates that part of the insects scored as moribund at 24 hours with this insecticide recovered at 48 hours. With Chlorpyrifos the opposite was observed, in other words, mortality increased from 24 hours to 48 hours.